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Elemental analysis of the thyroid by laser induced breakdown spectroscopy

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Abstract: The thyroid is an important hormone regulation organ. Laser induced breakdown spectroscopy (LIBS) is developed to assess iodine and other essential elements in the thyroid (of rats). Subjects are administered 0.05% iodine water for 0, 6, and 12 days before the thyroid is extracted. Pronounced iodine, sodium, calcium, and potassium emissions are observed at approximately 746, 589, 395/422, and 766/770 nm, respectively. Iodine emission is surprisingly highest in 0 day subjects, lowest after 6 days, and recovers by 12 days. This follows the Wolff–Chaikoff effect as ingestion of excess iodine reduces thyroid iodine and iodine is essential for hormone production. LIBS is a promising method for trace elemental analysis of the thyroid.

OCIS codes: (300.6365) Spectroscopy, laser induced breakdown; (170.6935) Tissue characterization.

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1. Introduction

The thyroid is an important endocrine gland located in the neck. It secretes hormones, such as triiodothyronine (T3) and thyroxine (T4), which are crucial for regulating metabolism and protein synthesis [1]. Metabolism and protein synthesis are essential for growth and development. Important elements in the thyroid include iodine (I), sodium (Na), calcium (Ca), and potassium (K). Iodine is a micronutrient that is essential for the production of T_3 and T_4 [2], which are partially composed of iodine. Improper levels of these elements and molecules can lead to a range of thyroid disorders, which include hypo and hyperthyroidism. The concentrations of these elements can also be distributed according to the Wolff–Chaikoff effect. The Wolff-Chaikoff effect is the observed reduction of thyroid hormone levels due to high iodine intake. It has been observed that reduced hormone levels occur with reduced thyroid iodine level. The mechanism for the acute Wolff–Chaikoff effect [3] is not completely understood, but is thought to be related to the generation of inhibitory substances on thyroid peroxidase activity [2]. Therefore, there is significant value in methodologies that can measure the levels of important elements within the thyroid which are involved with hormone production.

Laser induced breakdown spectroscopy (LIBS) is an elemental analysis method that uses a high intensity laser pulse to ablate the sample [4]. This leads to an optical emission spectrum that is characteristic of the elements in the sample along with their concentrations. LIBS has been applied to study soft tissues [5,6] such as the kidney, liver, and spleen. LIBS analysis of human nails has also been related to thyroid functioning [7,8]. In spite of the diverse nature of biological specimens, LIBS has become an effective analytical tool for biological applications, including in medicine [9,10]. The success of LIBS is due in large part to relatively compact and inexpensive instrumentation and easy sample preparation. Measurements can be performed in standard air atmosphere within seconds for in situ tissue characterization and multi-element analysis [11–13]. LIBS is also well suited to performing elemental analysis of micron level structures [5,14]. In comparison to similar elemental analysis methods such as x-ray fluorescence spectroscopy and laser ablation inductively coupled plasma mass spectroscopy, LIBS offers reduced sample preparation time, improved spatial resolution, increased sensitivity for light elements, and/or more portable instrumentation.

In this article, we develop LIBS to analyze many of the important elements in the rat thyroid in situ, including I, Na, Ca, and K. This initial study analyzes the thyroid at the organ

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level, but long-term, LIBS can be developed to perform cellular level elemental analysis. This will complement molecular analyses of thyroid cells [15–17].

2. Material and methods

2.1 Experimental setup

Figure 1 shows the setup of LIBS. The 1064 nm pulsed laser (CFR200, Quantel) emitted 8 ns, 200 mJ pulses focused to a 10 µm spot on the thyroid sample. The optical emission from the ablated tissue was collected by a six channel fiber (2000 µm diameter) bundle positioned 35 mm from the focus point and at 45° from the laser beam. The fibers relayed light to six spectrometers spanning 200 – 900 nm with 0.1 nm resolution (MX2500+ , Ocean Optics). The spectrometer was triggered to acquire 0.9 µs after laser firing and with 1 ms acquisition. LIBS was performed in standard atmospheric air. The computer processed the spectrum acquired by the spectrometers and produced the graphical presentation of spectral intensity against the corresponding wavelength.

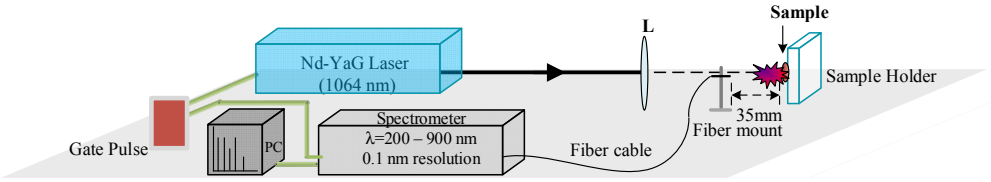

Fig. 1. Schematic diagram of the laser induced breakdown spectroscopy (LIBS) setup. The laser pulse is focused by the lens (L) onto the sample. The ablated portion of the sample emits light which is collected by the fiber and channeled to the spectrometer. The setup is controlled by a personal computer (PC), which triggers both the laser and the spectrometer, and displays the spectra.

2.2 Animal subjects

This study was approved by the animal research ethics committees of the City University of Hong Kong, the University of Hong Kong, and the Department of Health of the Hong Kong Special Administrative Region. Male Sprague Dawley (SD) rats (N = 30, 350 – 450 g) were employed. Subjects were provided by the AAALAC accredited Laboratory Animal Unit of the University of Hong Kong. Five subjects were housed in one cage under a constant 25 °C temperature and 60 to 70% humidity at the Laboratory Animal Research Unit of the City University of Hong Kong. Subjects were housed in 12/12 hour light/dark cycles with access to food and drinking water, ad libitum. Subjects were acclimated to the housing environment for at least one day prior to the experiment.

2.3 Iodine solutions

For the high iodine diet, molecular iodine was bought from Sigma Aldrich (USA). All chemicals and solvents used in this study were of analytical grade and obtained through commercial sources. The 0.05% solution was prepared by adding 1 g of crystal molecular iodine (solute) in 2000 mL of de-ionized water (solvent). To make the solute completely dissolved in solvent, magnetic stirring was performed in a light sensitive and sealed beaker for 48 hours at room temperature. Each time a fresh 0.05% iodine solution was prepared, it was placed in light sensitive 500 ml bottles and transferred to the cages for feeding the subjects. Following the same procedure, 0, 500 and 1000 part per million (ppm) iodine solutions were prepared for immersing excised thyroids. This was used to quantify the increase in LIBS signal due to increased thyroid iodine concentration.

2.4 Sample preparation

The 30 subjects were divided into six groups of five each. The six groups were split into two sets of three each. For the first set, two groups drank 0.05% iodine solution to model a high

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iodine diet for six and twelve days, respectively. On the 6th or 12th day, subjects were euthanized by 1 mL/kg body weight of 20% Dorminal Tropfen via intraperitoneal injection. The third group in the set, control subjects, were also sacrificed using the same method. The thyroid, which is located superior to the trachea (Fig. 2(a)), was extracted from the subject as shown in Fig. 2(b). All thyroids were immersed in saline to remove the existing blood. The organs were then placed in a 50 mL test tube and immersed in liquid nitrogen for several seconds followed by storage in a −80 °C freezer for 24 hours before performing LIBS. The second set of subjects were also sacrificed in the same way and the thyroids were rinsed with saline. This set was used to quantify the increase of LIBS signal with thyroid iodine concentration. The three groups were immersed in 0, 500 and 1000 ppm iodine solution for 24 hours at room temperature. After immersion, the thyroids were rinsed with saline and immersed in liquid nitrogen for several seconds followed by 24 hours storage in −80 °C before performing LIBS.

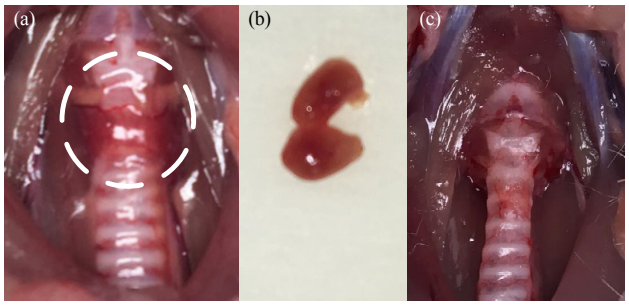

Fig. 2. (a) The portion of the neck superior to the trachea where the thyroid is located (circled). (b) The harvested thyroid sample showing both lobes. (c) The same portion of the neck after the thyroid was removed.

2.5 Data acquisition

One day prior to LIBS, samples were transferred from −80 °C and stored at −20 °C on a glass slide. The two lobes of the thyroid were placed at pre-marked positions on the slide to facilitate rapid sample positioning at the laser focus. At the time of acquisition, the slide was placed on the sample holder with the right lobe at the focus, three laser pulses were fired, and the emission spectra recorded. The slide was then moved to put the left lobe at the focus point and three more acquisitions were performed. The two slide positions on the holder were pre-marked and the laser and spectrometer were computer controlled to expedite data acquisition. The entire process of LIBS acquisition was completed approximately 60 s after removing from −20 °C to reduce sample warming.

2.6 Data analysis

The raw data from the spectrometer was imported to OriginLab software for data analysis. To subtract background from data, a baseline correction was performed. To calibrate the wavelength measurement, the spectrometer was calibrated using the Argon gas spectrum. The Argon spectrum was matched with Argon spectral lines available in the Atomic Spectra Database of the National Institute of Standards and Technology (NIST). Calibrated spectra were also matched with the database for elemental analysis.

The spectra from the six laser pulses fired at each thyroid were averaged to obtain the spectrum for the thyroid. The intensity of the entire spectrum was then normalized by that of the 656.2 nm hydrogen line. Statistical analysis of the spectra was performed with Microsoft Excel. T-tests were performed across the subject groups using the two-tailed distribution and unpaired t-testing. A p-value threshold of 0.05 was considered statistically significant. Linear regression analysis was also applied to the iodine solution immersed thyroid data to compute R^2 and demonstrate positive correlation between LIBS signal and iodine concentration.

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3. Results and discussion

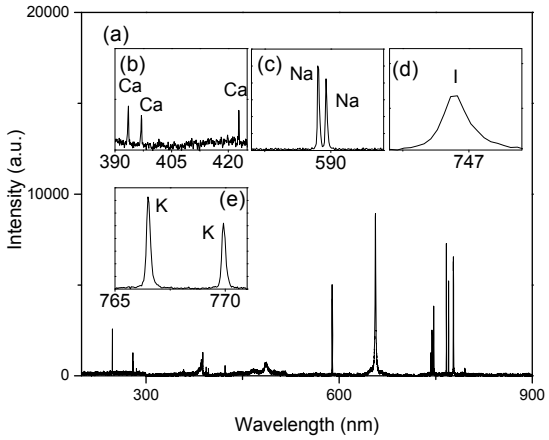

Fig. 3. (a) LIBS spectrum from the thyroid of a control subject. (b) Calcium (Ca) emission lines are observed at 393.4, 396.9 and 422.7 nm. (c) Sodium (Na) lines are at 589.0 and 589.5 nm. (d) Iodine (I) line is at 746.9 nm. (e) Potassium (K) lines are at 766.4 and 769.9 nm.

Figure 3 shows the LIBS spectrum obtained from the thyroid of a control subject. Figure 3(a) shows the whole spectrum from 200 to 900 nm. There are several prominent emission lines showing the spectral signature of different elements. The sub-panels, Figs. 3(b)-3(e) show the spectrum expanded about different wavelength ranges. Calcium lines are observed at 393.4, 396.9 and 422.7 nm. Sodium lines are observed at 589.0 and 589.5 nm. An iodine line is observed at 746.9 nm. Potassium lines are observed at 766.4 and 769.9 nm. These are important elements for proper biological function. A hydrogen line is also observed at 656.2 nm, which will be used to normalize spectra from different samples. Amongst the key trace elements, K lines have the highest intensity, followed by Na and I, while Ca has the lowest intensity. The unnormalized intensities (mean ± standard deviation) of the six shots from one control subject (Fig. 3) for Ca, Na, I, K, and H are 460.3 ± 193.7, 4533 ± 1033, 3761 ± 1590, 6245 ± 2654 and 8818 ± 2004, respectively.

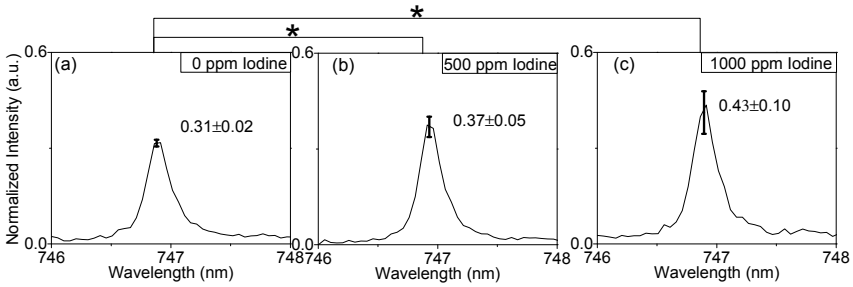

Fig. 4. (a) Group averaged iodine emission line at 746.9 nm acquired from thyroids (N = 5) immersed in 0 ppm iodine solution (saline only). The intensity has been normalized by that of the hydrogen line at 656.2 nm. (b and c) Iodine lines from thyroids immersed in 500 ppm (N = 5) and 1000 ppm (N = 5) solutions, respectively. Statistical testing was performed with a standard two-tailed t-test. * indicates $p < 0.05$. Error bars indicate standard deviation of intensity at 746.9 nm. Higher iodine concentration in the thyroid leads to higher LIBS intensity.

Figure 4(a) shows the group averaged iodine emission line at 746.9 nm acquired from thyroids immersed in 0 ppm iodine solution for 24 hours. In comparison, Fig. 4(b) shows the iodine line from thyroids immersed in 500 ppm solution. The difference in normalized intensity between iodine lines after 0 and 500 ppm immersion is found to be $\Delta I = 0.06$. This difference is statistically significant. Therefore, immersing the thyroid in 500 ppm iodine

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solution increases the intensity of the iodine emission line. Similarly, Fig. 4(c) shows the iodine line after immersing the thyroids in 1000 ppm solution. The difference in intensity between 0 and 1000 ppm lines is ΔI = 0.12. The 0 and 1000 ppm lines are also statistically significantly different. The difference in intensity between 500 and 1000 ppm lines is ΔI = 0.06. The linear regression analysis shows a strong positive correlation (R^2 = 0.65) between LIBS intensity and iodine concentration. This result demonstrates that the 746.9 nm line belongs to iodine and that higher LIBS intensity corresponds to higher iodine concentration. Note that these concentrations are close to, but above physiological values as the normal thyroid (0 ppm) has iodine concentration in excess of 1000 ppm [18,19].

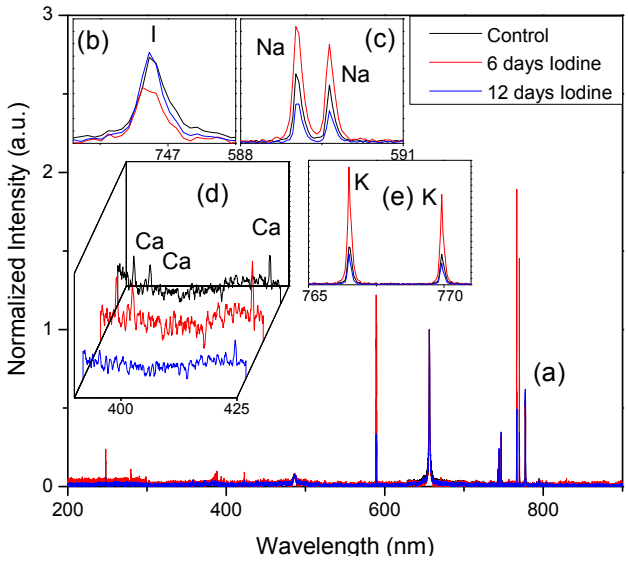

Fig. 5. (a) Normalized and group averaged LIBS spectra from the thyroids of control subjects (N = 5), subjects treated for 6 days with 0.05% iodine solution (N = 5), and subjects treated for 12 days (N = 5). (b-e) Spectra expanded about the I, Na, Ca and K lines, respectively. A high iodine diet initially reduces thyroid iodine concentration before recovery. This is in agreement with the Wolff–Chaikoff effect. The concentrations of other elements are also affected by high iodine intake.

Figure 5(a) shows the normalized LIBS spectra from the thyroids of control subjects, subjects treated for 6 days with 0.05% iodine solution, and subjects treated for 12 days. Figure 5(b) shows the emission line of Iodine. The thyroid iodine concentration is highest in controls. Interestingly, after 6 days of high iodine intake, the iodine concentration decreases. The reduction is statistically significant (see Fig. 6). After 12 days of high iodine intake, the iodine concentration approaches that of the controls ($p < 0.05$ when comparing 6 days with 12 days and $p > 0.05$ when comparing controls with 12 days, see Fig. 6). This trend follows the hypothesis of the acute Wolff–Chaikoff effect. In rats, the Wolff-Chaikoff effect is associated with a marked decrease in expression of the sodium–iodide symporter (NIS) that is present on the basolateral membrane of thyroid follicular cells [20]. NIS mediates the active transport of iodine from the circulation into the thyroid [21]. The experimental investigation of the thyroid using LIBS likely demonstrates reduced iodine transport from the circulation into the thyroid. The 12 day treatment with observed recovery also agrees with the 10 day escape period from the Wolff–Chaikoff effect [20].

Figure 5(c)-5(e) show the emission lines of Na, Ca and K, respectively. With the decrease in thyroid iodine concentration following 6 days high iodine intake, Na, Ca, and K increase (not statistically significant). With recovery of iodine after 12 days, Na, Ca, and K decrease towards control levels. The change in concentration of these elements with iodine excess can result in adverse thyroidal effects and decreased synthesis of thyroid hormones [22–24].

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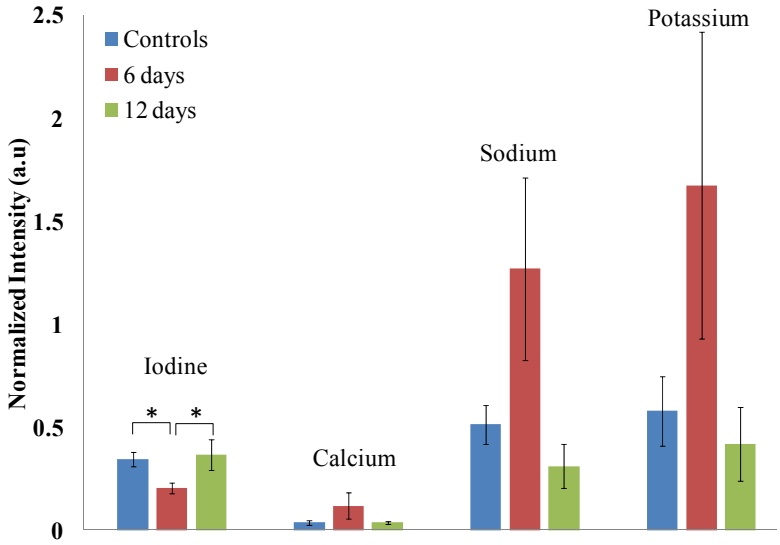

Fig. 6. Barplot showing the statistical analysis of the LIBS spectra from control, 6 day iodine treated and 12 day treated thyroids. Mean ± standard deviation (error bars) are shown for iodine, calcium, sodium and potassium. For elements with more than one measured emission line, the intensities were averaged. Standard two-tailed t-testing was used to compare normalized emission lines between the three groups. * indicates $p < 0.05$.

4. Summary

Laser induced breakdown spectroscopy is developed to assess iodine in the thyroid in situ, along with other elements such as sodium, calcium, and potassium. We also demonstrate the hypothesis of Wolff–Chaikoff. The investigation of the thyroid in control and iodine treated subjects shows a marked reduction in intrathyroidal iodine following increased iodine intake. Further, the Wolff–Chaikoff effect duration agrees with the recovery period in which iodine intensity increases with supplemented iodine. This new method will help reveal the underlying mechanisms of iodine-induced thyroid disorders. The LIBS approach provides a novel method for investigating the endocrine system.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.